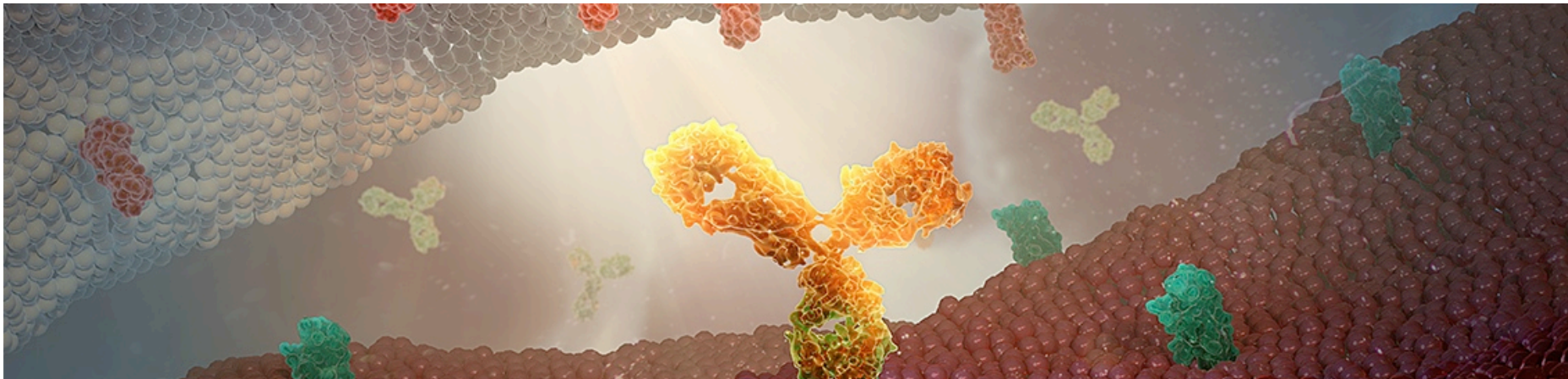


Does platform matter for ADA assessment? Validation and sample data revisited across multiple platforms

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16th November 2017



Overview



ADA as a bioanalytical challenge



Initiating a comparison of platforms



Challenges and conclusions of development



Comparison of test sample data

Meeting regulatory expectations for assessment of ADA is a fundamental bioanalytical challenge

- Immunogenicity is a critical component of drug development
- Agency expectations for assessment of ADA have developed significantly over the past decade and continue to challenge
- Assay validation packages are submitted at license application (potentially earlier)
 - Methods are not deemed validated until the regulators review and agree
- Strategic decisions are not made lightly – and that includes choice of platform

The image displays two regulatory documents side-by-side. The left document is titled 'Assay Development and Validation for Immunogenicity Testing of Therapeutic Protein Products' and is a 'DRAFT GUIDANCE' from the U.S. Department of Health and Human Services, Food and Drug Administration. The right document is titled 'Guideline on Immunogenicity assessment of biotechnology-derived therapeutic proteins' and is a 'Draft' from the European Medicines Agency. Both documents include sections for 'Comments and suggestions' and 'Comments'.

Assay Development and Validation for Immunogenicity Testing of Therapeutic Protein Products
Guidance for Industry
DRAFT GUIDANCE

This guidance document is being distributed for comment purposes only. Comments and suggestions regarding this draft document should be submitted within 60 days of publication in the Federal Register of the notice announcing the availability of the draft guidance. Submit electronic comments to www.fda.gov/oc/ohrt. Submit written comments to the Division of Dockets Management (HFA-305), Food and Drug Administration, 10155 Beltsville Lane, rm. 105, Beltsville, MD 20705. All comments should be identified with the docket number listed in the notice of availability that publishes in the Federal Register.

For questions regarding this draft document, contact CDER's Office of Communications and Public Affairs (CDER/OCPA) at (301) 443-1771.

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research
Center for Biologics Evaluation and Research
Center for Devices and Radiological Health

April 2016
Pharmaceutical Quality/CAC
Revision 1

EUROPEAN MEDICINES AGENCY
SCIENCE. INTEGRITY. SERVICE

24 September 2015
(EMA/CMP/001/14/12/2016, Rev. 1)
Guideline for Therapeutic Protein Products (TTPPs)

Guideline on Immunogenicity assessment of biotechnology-derived therapeutic proteins
Draft

Event	Date
Draft agreed by Biotechnological Products Working Party (BPWP)	August 2015
Adopted by CHMP for release for consultation	24 September 2015
Start of public consultation	01 October 2015
End of consultation (deadline for comments)	31 January 2016

The guideline replaces 'Guideline on immunogenicity assessment of biotechnology-derived therapeutic proteins' (EMA/CMP/001/14/12/2016, Rev. 1).

Comments should be provided using this [link](http://www.ema.europa.eu/ema). The completed comments form should be sent to ema@ema.europa.eu.

Keywords
Immunogenicity, therapeutic proteins, anti-drug antibodies (ADA), assays, assay design, binding antibodies, neutralizing antibodies, risk, safety, efficacy, pharmacokinetics, risk management, integrative summary of immunogenicity.

EMA/CP/001/14/12/2016, Rev. 1
EMA/CP/001/14/12/2016, Rev. 1
EMA/CP/001/14/12/2016, Rev. 1



Specific instruments become preferred platforms for different applications



Platform	PK	PD	ADA
Gyros xP	✓	✓	
MSD S 600	✓	✓	✓
Molecular Devices Paradigm	✓	✓	

✓ = primary platform

✓ = alternate platform

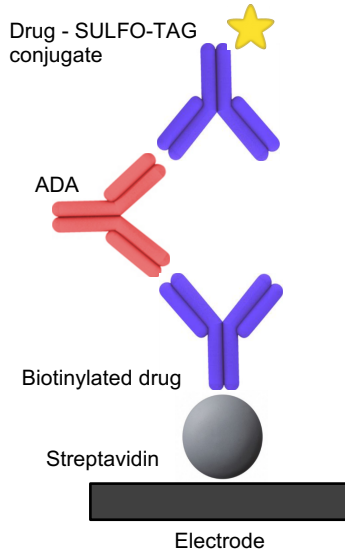
....But relying on a single vendor is potentially high-risk

- Bioveris remains a cautionary lesson
- New challenges may be better solved on alternate platforms
 - Moving beyond mAbs
 - Conjugated molecules
 - Multiple domains

A platform comparison of MSD versus Gyrolab and AlphaLISA technologies was initiated

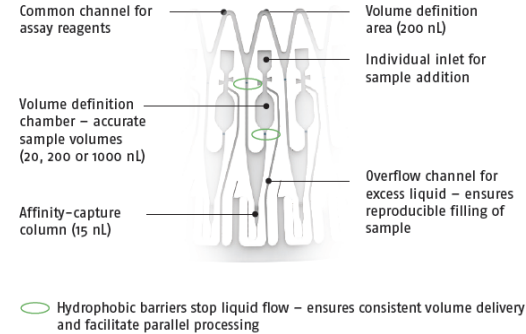
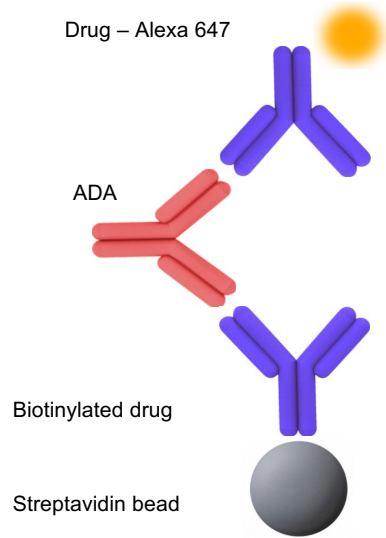


The MSD has gained significant traction in industry for analysis of immunogenicity



- Electrochemiluminescent immunoassay
 - Immunoassay complex is built on carbon electrode
 - Ruthenylated detection reagent in proximity of the electrode emits light in presence of TPA substrate in Read Buffer
- Carbon electrode has ~10x greater binding capacity than polystyrene
- Signal amplification from multiple levels of excitation per label
- Typically offers increased sensitivity and drug tolerance over ELISA

Gyrolab is a semi-automated platform using nano-fluidics on a CD micro-laboratory

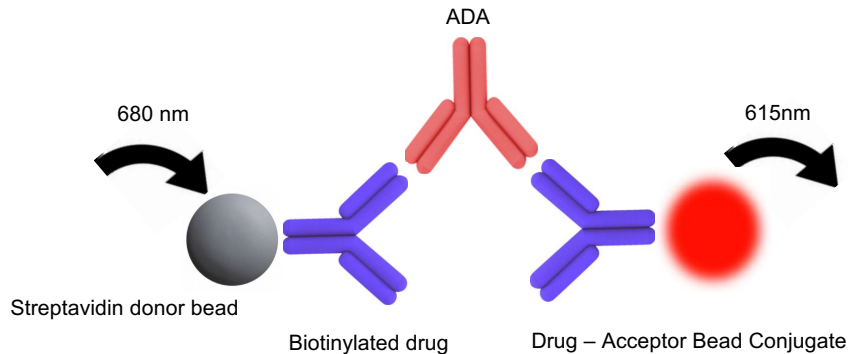


© Gyros Protein Technologies

- Gyrolab ADA Solution
 - Bioaffy 200 CD
 - Bioaffy Mixing CD for acid-dissociation
 - Rexpix ADA Assay Buffer
- Updated this year to include 96 micro-laboratory mixing CD and updates to software

AlphaLISA is a bead-based no-wash solution phase immunoassay

- Amplified Luminescent Proximity Homogenous Assay
 - Solution phase assay
 - Laser excitation of donor bead leads to release of singlet oxygen molecules triggering energy transfer and spike of specific light emission from acceptor beads



- No wash protocol
 - Theoretically beneficial for detection of low affinity ADA

Method development followed a standard approach across the platforms

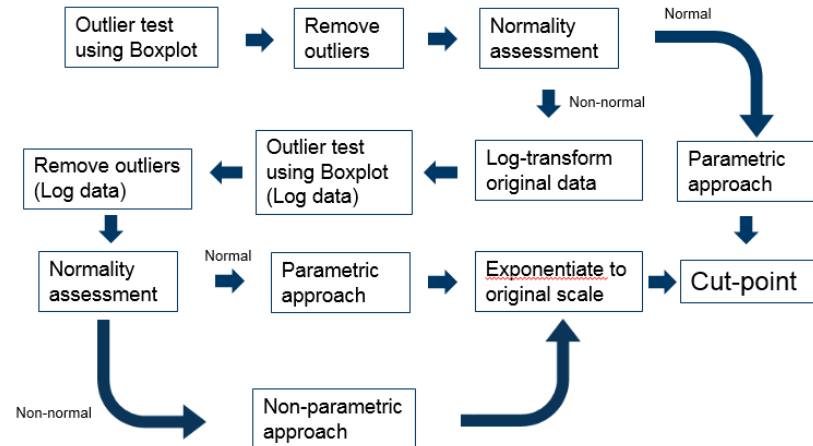
Reagent labelling

Chequerboard and assessment of MRD

Assessment of sensitivity and early look at cut-point

Assessment of drug tolerance

Cut-point assessment
N=30 individual samples



Workflows highlight operator efficiencies for Gyrolab and AlphaLISA over MSD

Day 1

MSD

Prepare samples (1:10 MRD)

Prepare mastermix

Mix samples and mastermix

Incubate o/n at RT on shaker

Block Streptavidin plate o/n

Gyrolab

Centrifuge samples/reagents

Prepare samples (1:4 MRD)

Prepare mastermix

Mix samples and mastermix

Incubate o/n at 2 to 8°C

AlphaLISA

Prepare samples (1:20 MRD)

Prepare mastermix

Mix samples and mastermix

Incubate o/n at RT on shaker

Day 2

Streptavidin plate wash

Sample transfer

Incubate 1 hour at RT on shaker

Wash plate

Add Read Buffer and read plate

Load plate on xP and read

Add Donor Beads

Load plate on Paradigm and read



Platform-specific strengths and weaknesses are evident for each platform

Platform	Pros	Cons
MSD	<ul style="list-style-type: none">• Read Time ~90 seconds• Open reagent choice enables custom optimisation• Cost	<ul style="list-style-type: none">• Relatively labour intensive
Gyrolab	<ul style="list-style-type: none">• Gyrolab Evaluator Software• Efficient workflow• Choice of off-the-shelf reagents• Application support	<ul style="list-style-type: none">• Read Time ~50 mins• Sample handling requirements – impact on precision• Closed reagents limit custom optimisation
AlphaLISA	<ul style="list-style-type: none">• Efficient workflow• Read Time ~120 seconds	<ul style="list-style-type: none">• Buffer selection challenging• Additional development steps to optimise biotin Ab and bead concentrations• Expense of beads



Assay parameters indicate comparable performance between MSD and Gyrolab

	MSD	Gyrolab	AlphaLISA
Reagent Labelling	Biotin and SULFO-TAG 12:1	Biotin and Alexa 647 12:1	Biotin and Donor Beads 12:1 and 50:1
Mastermix	2.5 µg/mL Biotin 2.5 µg/mL SULFO-TAG	4 µg/mL Biotin 4 µg/mL Alexa	2 nM Biotin 10 µg/mL Acceptor 40 µg/mL Donor
MRD	1:10	1:4	1:20
Assay Buffer	1% Blocker A	Rexxip ADA	HEPES/Casein/Tween
Hook Effect	Not evident at 50,000 ng/mL	Not evident at 50,000 ng/mL	Not assessed
Precision	≤ 9.9% CV	≤ 17.5% CV	Not assessed
Sensitivity	193 ng/mL	168 ng/mL	Not assessed
Drug Tolerance	500 ng/mL PC tolerant of 10 µg/mL drug*	500 ng/mL PC tolerant of 10 µg/mL drug*	Not assessed
Cut-point Factor	1.22	2.70	Not assessed

*tolerant to between 10 µg/mL and 100 µg/mL of therapeutic



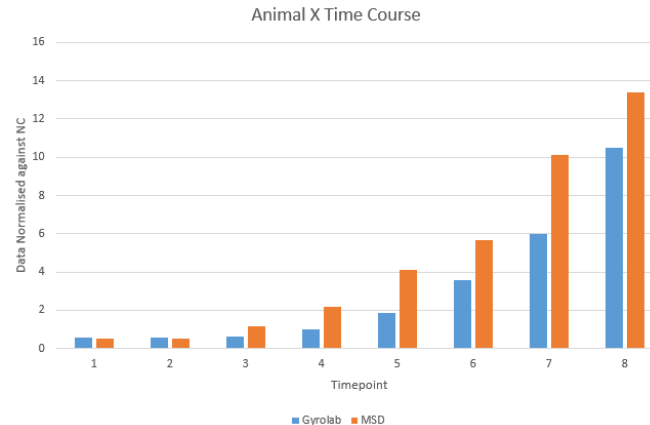
Sample analysis highlighted a need for further development on the Gyrolab

- Six Month Repeat Dose TK Study with 16 Week Treatment-Free Period
 - Four dose groups:
 - Group 1 Control
 - Group 2 50 mg/kg SC
 - Group 3 150 mg/kg SC
 - Group 4 150 mg/kg IV
- 266 samples reanalysed over 6 CD's over 2 days
- **Assay failure → Unacceptable precision from PC samples
→ Clearly erroneous data around low end of the assay**

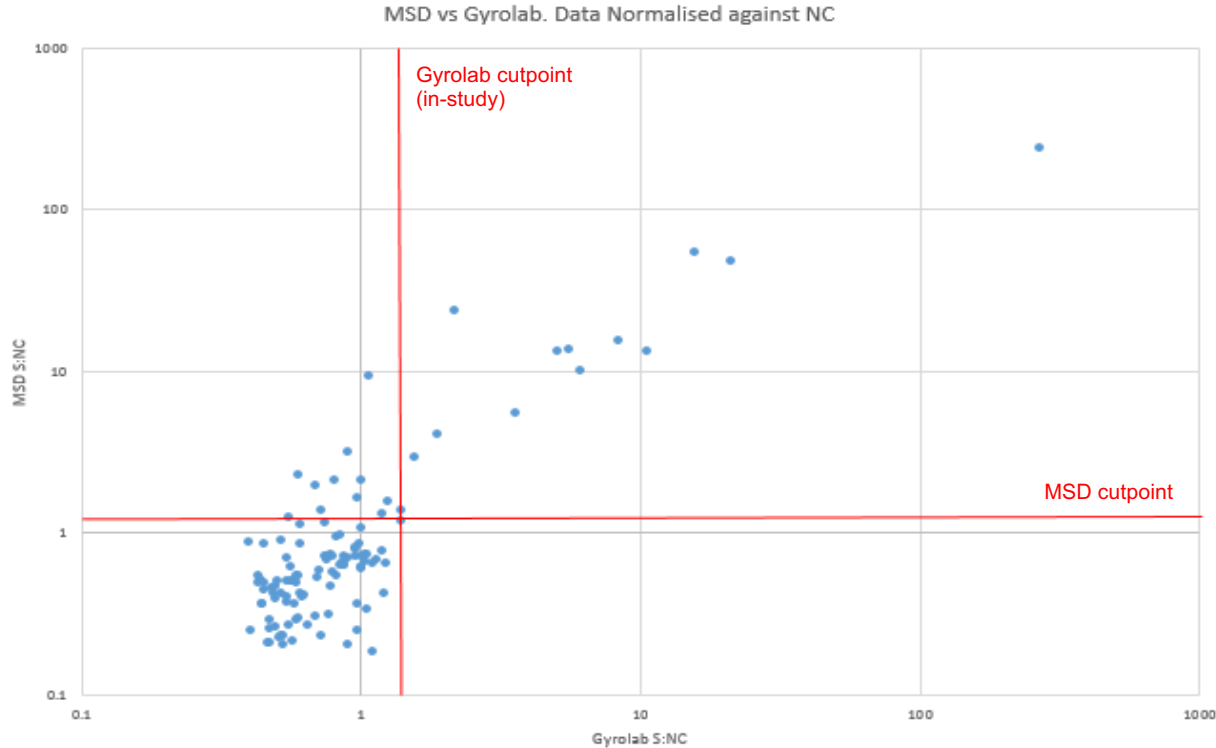


Rexxip ADA was substituted for Rexxip F to increase stringency

- **Development is ongoing**
 - 3 CDs of TK samples reanalysed to aid troubleshooting
 - Available data indicate increased stringency and improved assay performance
 - Positive control data show precision $\leq 9.1\%$ CV
- Preliminary comparison against MSD data possible and some interesting correlation appears



Plotting normalised data indicates MSD detects more positive samples



- MSD detected 4 false positives
- Gyrolab detected 1 false positive
- Gyrolab PC at 50 ng/mL would be above estimated cut-point



Conclusions

- Immunogenicity assessment remains a key bioanalytical challenge
- Committing to a single platform can seem the safest option
- There remains a desire to 'stay current' with alternate technologies
- Comparison of platforms highlights process, cost and data as decision making variables
- There is a need to develop a significant expertise in each platform to appropriately optimise assays and thus negate data as a variable



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